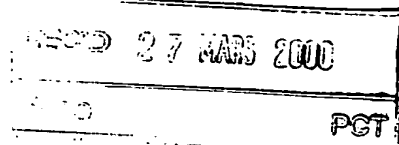


## PATENT COOPERATION TREATY

## PCT





## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PCT/ST-22	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/IT98/00364	International filing date (day/month/year) 16/12/1998	Priority date (day/month/year) 19/12/1997
International Patent Classification (IPC) or national classification and IPC C07K14/47		
Applicant SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.  <input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  These annexes consist of a total of 6 sheets.
3. This report contains indications relating to the following items:  I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input checked="" type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application

Date of submission of the demand 14/07/1999	Date of completion of this report 21.03.00
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Armandola, E  Telephone No. +49 89 2399 7493

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IT98/00364

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

### Description, pages:

1-11	as originally filed	
12	with telefax of	04/02/2000

### Claims, No.:

1-12	with telefax of	04/02/2000
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### Drawings, sheets:

1/6-4/6	as originally filed	
5/6,6/6	with telefax of	04/02/2000

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/IT98/00364

- ☒ claims Nos. (4, 9) partially (N, IS, IA); 6-8 (IA).

because:

- ☒ the said international application, or the said claims Nos. 6-8 (IA) relate to the following subject matter which does not require an international preliminary examination (*specify*):

**see separate sheet**

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

- ☒ the claims, or said claims Nos. (4, 9) partially are so inadequately supported by the description that no meaningful opinion could be formed.

- ☐ no international search report has been established for the said claims Nos. .

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.  
☐ paid additional fees.  
☐ paid additional fees under protest.  
☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☒ complied with.  
☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/IT98/00364

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- ☒ all parts.
- ☐ the parts relating to claims Nos. .

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-12
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-5, 9-12
	No:	Claims	

**2. Citations and explanations**

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Novelty, Inventive step and Industrial applicability

The part of Claims 4 and 9 referring to inflammatory diseases was not examined due to the lack of disclosure regarding this part of the claims (see Item VIII, 1) ii) below).

Industrial applicability

Claims 6-8 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34(4)(a)(i) PCT).

For the assessment of the present Claims 6-8, with regard to methods of treatment of the human body, on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claim. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

**Re Item IV**

**Lack of unity of invention**

The objection to lack of unity raised by the ISA is not maintained by this authority. Therefore, all parts of the present application were subjected to International Preliminary Examination (IPE).

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/IT98/00364

Reference is made to the following document:

D1: VOURET-CRAVIARI V ET AL: 'Expression of a long pentraxin, PTX3, by monocytes exposed to the mycobacterial cell wall component lipoarabinomannan.' INFECTION AND IMMUNITY, (1997 APR) 65 (4) 1345-50. , XP002113122

Prior art

Document D1 studies the expression of mouse PTX3 upon stimulation with mycobacterial components. It suggests a role for PTX3 in mycobacterial infections but does not disclose the possible use of PTX3 in therapy of microbial infections.

Novelty and Inventive step (Art. 33(3)(4) PCT)

The subject matter of claims 1-12 can be considered novel and inventive because neither pharmaceutical compositions, nor methods of gene therapy for the treatment of tumor conditions involving the use of PTX3 nor the use of the cDNA corresponding to PTX3 in the preparation of medicaments are disclosed or suggested in the prior art.

D3 discloses that IL-1, TNF, lipopolysaccharide and mycobacterial cell wall components induce the expression and production of the long pentraxin PTX3, but does not suggest that long pentraxin PTX3 can have a therapeutic effect on infectious diseases, or on tumor conditions.

Although the claims are considered novel and inventive, it should be noted that for the subject-matter of claims 4-12 it seems that the invention is not disclosed in a manner sufficiently clear and complete to be carried out by the person skilled in the art (Art. 5 PCT) and that is not fully supported by the description (Art. 6 PCT) (see Item VIII).

**Re Item VIII**

**Certain observations on the international application**

Sufficiency of disclosure and support by the description (Art.5, Art. 6 PCT)

i) With regard to Claims 4, 6-10 and 12, in reference to the use of PTX3 in the treatment of tumors, the claims are not considered to be sufficiently disclosed nor supported by the

description.

Claim 4 refers to a composition containing the PTX3 protein for the treatment of tumors: such a composition is not disclosed in the description. Instead a composition containing PTX3, useful for the recruitment of leukocytes is disclosed (see p.8). The fact that such a composition can induce leukocyte recruitment is far from indicating its usefulness in treating tumor conditions, and the skilled person would find no indication in the description that might imply so. The same holds true for Claims 9 and 10, referring to the use of the PTX3 protein for preparing a medicament for treating tumor disease and for Claim 12 referring to the use of the PTX3 DNA for the same purpose.

Claim 6 concerns a method for the treatment of an established tumor. On page 9 of the description, one example of the possibility to prevent *in vivo* proliferation of a murine mastocytoma by using PTX3 is given. In the experiment healthy mice are injected with P815 tumor cells transfected with PTX3 cDNA and tumor rejection is observed. This experiment cannot be seen as a basis for the method described in Claim 6.

The same is true for Claims 7 and 8 in which a method of gene therapy using a retroviral vector for the treatment of tumors is claimed. Such method is not disclosed in the description and is not supported by any example.

ii) Claims 4, 5 and 9-11 refer to the treatment of infectious diseases caused by bacteria, fungi, protozoa or viruses.

The prior art (D1) teaches that PTX3 is induced on monocytes by mycobacterial cell wall component and LPS; D1 suggests a possible use of long PTX3 as a diagnostic agent for infections, it does not teach or even suggests that long PTX3 can have a therapeutic effect on infectious diseases.

The application does not prove that PTX3 could be useful for the therapy of infectious diseases, and the only example that might support this notion is the leukocyte recruitment data obtained by injection of PTX3.

It is, however, well known that resistance or susceptibility to a certain microorganism, as well as the ability to successfully fight an infection rather than developing a chronic form of the disease, are linked to the quality of the immune response developed by the organism (e.g. Th1 vs. Th2 induction). The fact that PTX3 induces leukocyte recruitment does not imply that it can act as a therapeutic agent for infectious diseases, as this may depend on the type of cytokines released by the recruited leukocytes upon contact with the microorganism.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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iii) In Claims 4, 9 and 10 reference is made to inflammatory diseases as targets of treatment with PTX3.

As no proof that might support the usefulness of PTX3 in treating inflammatory diseases can be found in the description, the notion that PTX3 might have a role in inflammatory diseases is of purely speculative nature (see also Item III).



## INTERNATIONAL SEARCH REPORT

National Application No  
PCT/IT 98/00364

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/47 C12N15/12 A61K38/17 C12N15/10 C12P21/02  
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOTTAZZI, B. (1) ET AL: "Preliminary biochemical and biological characterization of ptx3, a new member of the pentraxin gene family." CYTOKINE, (NOV., 1997) VOL. 9, NO. 11, PP. 903. MEETING INFO.: FIFTH ANNUAL CONFERENCE OF THE INTERNATIONAL CYTOKINE SOCIETY LAKE TAHOE, NEVADA, USA NOVEMBER 9-13, 1997 INTERNATIONAL CYTOKINE SOCIETY. , XP002113120 the whole document ---	1-5, 12-18
X	INTRONA M ET AL: "Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites." BLOOD, (1996 MAR 1) 87 (5) 1862-72. , XP002113121 the whole document ---	6-11
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*Z\* document member of the same patent family

Date of the actual completion of the international search

24 August 1999

Date of mailing of the international search report

03/09/1999

Name and mailing address of the ISA

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Moreau, J

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/IT 98/00364

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 21313 A (ITALFARMACO) 28 October 1993 (1993-10-28)	6-11
A	the whole document	1-5, 12-18
A	----- VOURET-CRAVIARI V ET AL: "Expression of a long pentraxin, PTX3, by monocytes exposed to the mycobacterial cell wall component lipoarabinomannan." INFECTION AND IMMUNITY, (1997 APR) 65 (4) 1345-50. , XP002113122 the whole document -----	1-18

## PCT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
 United States Patent and Trademark  
 Office  
 Box PCT  
 Washington, D.C.20231  
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 19 October 1999 (19.10.99)	
International application No. PCT/IT98/00364	Applicant's or agent's file reference PCT/ST-22
International filing date (day/month/year) 16 December 1998 (16.12.98)	Priority date (day/month/year) 19 December 1997 (19.12.97)
Applicant BOTTAZZI, Barbara et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:  
 14 July 1999 (14.07.99)

☐ in a notice effecting later election filed with the International Bureau on:  
 \_\_\_\_\_

2. The election ☒ was  
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Maria Victoria CORTIELLO Telephone No.: (41-22) 338.83.38
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**Brief description of drawings**

**Figur 2:** PTX3 binding to C1q. Panel A shows the binding of the supernatant of the culture containing PTX3 (sense) and of the purified protein to C1q and C1s immobilised on plate. The binding is assessed as optical density (O.D.) at 405 nm. Panel B shows the saturation curve obtained with the biotinylated protein. The kinetic parameters were calculated using the non-linear fitting statistical method.

**Figure 3:** PTX3-induced leukocyte recruitment: 1 µg of PTX3 is injected into a subcutaneous pocket induced in the back of CD1 mice by inoculation of 5 ml of air.

**Figure 4:** PTX3-induced leukocyte recruitment in normal animals and in genetically modified animals without C1q. PTX3 is injected into a subcutaneous induced on the back of the animals.

**Sequence 1:** Amino acid sequence of human PTX3. The underlined amino acids constitute the peptide signal. Mature hPTX3 consists of 364 amino acids.

**Sequence 2:** Nucleotide sequence of fragment of cDNA of human PTX3. Upper case letters denote nucleotides coding for the protein, while lower case letters denote regions at 3' and 5' not translated but present in the construct.

**CLAIMS**

1. Orally, parenterally, transdermally or subcutaneously administrable pharmaceutical composition containing as active ingredient the amino acid sequence of the long pentraxin PTX3, and a pharmacologically acceptable excipient.
2. Composition according to claim 1, in which the sequence of the long pentraxin PTX3 is the sequence of naturally occurring PTX3.
3. Composition according to claim 2, in which the sequence of the long pentraxin PTX3 is the sequence of human PTX3.
4. Composition according to claims 1-3, for the treatment of infectious and inflammatory diseases or tumours.
5. Composition according to claim 4, for the treatment of diseases caused by bacteria, fungi, protozoa or viruses.
6. Expression vector containing the complete cDNA sequence coding for the long pentraxin PTX3 or one of its functional derivatives.
7. Vector according to claim 6, which is a plasmid.
8. Vector according to claim 7, which is the plasmid pSG5.
9. Recombinant host cells transfected with the expression vector of claims 6-8.
10. Host cells according to claim 9, which are CHO cells.
11. Method for producing long pentraxin PXT3 or one of its functional derivatives comprising the culturing of the cells of claims 9-10.

12. Method of gene therapy for the treatment of tumour conditions, comprising:

- (a) taking samples of cells from a patient suffering from a cancer;
- (b) transfecting such cells with an expression vector containing  
5 the complete cDNA sequence coding for the long pentraxin  
PTX3 or one of its functional derivatives; and
- (c) inoculating said patient with such transfected cells.

13. Method of gene therapy for the treatment of tumour conditions comprising:

- 10 a) preparation of an expression vector of viral origin containing  
the complete cDNA sequence coding for the long pentraxin  
PXT3 or one of its functional derivatives; and
- b) injection of the expression vector thus obtained into a patient  
suffering from a cancer.

15 14. Method according to claim 13, in which the expression vector of  
viral origin is an adenovirus or retrovirus.

15. Use of the long pentraxin PTX3 for the preparation of a  
medicament for the treatment of infectious, inflammatory or  
tumour diseases.

20 16. Use according to claim 15, in which the long pentraxin PTX3 is  
the human pentraxin having sequence 1.

17. Use according to claim 16, for the preparation of a medicament for the treatment of diseases caused by bacteria, fungi, protozoa or viruses.
18. Use of cDNA coding for the long pentraxin PTX3 or one of its  
5 functional derivatives for the preparation of expression vectors containing such cDNA to be used in gene therapy methods for the treatment of tumour conditions.

Sequence 1

				<u>M</u>	1
<u>HLLAILFCAL</u>	<u>WSAVLAENSD</u>	DYDLMYVNLD	NEIDNGLHPT		41
EDPTPCDCGQ	EHSEWDKLF I	MLENSQMRER	MLLQATDDVL		81
RGELQRLREE	LGR LAESLAR	PCAPGAPAEA	RLTSALDELL		121
QATRDAGRRL	ARMEGAE AQR	PEEAGRALAA	VLEELRQTRA		161
DLHAVQGWAA	RSWLPAGCET	AILFPMRSKK	IFGSVHPVRP		201
MRLESFSACI	WVKATDVLNK	TILFSYGTKR	NPYEIQLYLS		241
YQSIVFVVGG	EENKLVAEAM	VSLGRWTHLC	GTWNSEEGLT		281
SLWVNGELAA	TTVEMATGHI	VPEGGILQIG	QEKNGCCVGG		321
GFDETLAFSG	RLTGFNIWDS	VLSNEEIRET	GGAESCHIRG		361
NIVGWGVTEI	QPHGGAQYVS				381



Sequence 2

	ctca aactcagctc acttgagagt ctcctccccgc cagctgtgga aagaactttg	54
5	cgtctctcca gcaATGCATC TCCTTGCGAT TCTGTTTTGT GCTCTCTGGT CTGCAGTGT	114
	GGCCGAGAAC TCGGATGATT ATGATCTCAT GTATGTGAAT TTGGACAACG AAATAGACAA	174
	TGGACTCCAT CCCACTGAGG ACCCCACGCC GTGCGACTGC GGTGAGGAGC ACTCGGAATG	234
	GGACAAGCTC TTCATCATGC TGGAGAAGTC GCAGATGAGA GAGCGCATGC TGCTGCAAGC	294
	CACGGACGAC GTCCTGCGGG GCGAGCTGCA GAGGCTGCGG GAGGAGCTGG GCCGGCTCGC	354
10	GGAAAGCCTG GCGAGGCCGT GCGCGCCGGG GGCTCCCGCA GAGGCCAGGC TGACCAGTGC	414
	TCTGGACGAG CTGCTGCAGG CGACCCGCGA CGCGGGCCGC AGGCTGGCGC GTATGGAGGG	474
	CGCGGAGGCG CAGCGCCCAG AGGAGGCGGG GCGCGCCCTG GCCGCGGTGC TAGAGGAGCT	534
	GCGGCAGACG CGAGCCGACC TGCACGCGGT GCAGGGCTGG GCTGCCCCGA GCTGGCTGCC	594
	GGCAGGTTGT GAAACAGCTA TTTTATTCCC AATGCGTTCC AAGAAGATT TTTGAAGCGT	654
15	GCATCCAGTG AGACCAATGA GGCTTGAGTC TTTTAGTGCC TGCATTTGGG TCAAAGCCAC	714
	AGATGTATTA AACAAAACCA TCCTGTTTTT CTATGGCACA AAGAGGAATC CATATGAAAT	774
	CCAGCTGTAT CTCAGCTACC AATCCATAGT GTTTGTGGTG GGTGGAGAGG AGAACAAACT	834
	GGTTGCTGAA GCCATGGTTT CCCTGGGAAG GTGGACCCAC CTGTGCGGCA CCTGGAATTC	894
	AGAGGAAGGG CTCACATCCT TGTGGGTAAA TGGTGAAGTG GCGGCTACCA CTGTTGAGAT	954
20	GGCCACAGGT CACATTGTTC CTGAGGGAGG AATCCTGCAG ATTGGCCAAG AAAAGAATGG	1014
	CTGCTGTGTG GGTGGTGGCT TTGATGAAAC ATTAGCCTTC TCTGGGAGAC TCACAGGCTT	1074
	CAATATCTGG GATAGTGTTT TTAGCAATGA AGAGATAAGA GAGACCGGAG GAGCAGAGTC	1134
	TTGTCACATC CGGGGGAATA TTGTTGGGTG GGGAGTCACA GAGATCCAGC CACATGGAGG	1194
	AGCTCAGTAT GTTTCAtaaa tgttgtagaa ctccacttga agccaaagaaa gaaactcac	1254
25	acttaaaaca catgccagtt gggaaggtct gaaaactcag tgcataatag gaacacttga	1314
	gactaatgaa agagagagtt gagaccaatc tttatttgta ctggccaaat actgaataaa	1374
	cagttgaagg aaagacattg gaaaaagctt	1404